DNA segment encoding the humanized immunoglobulin light chain variable region into the cell,

wherein the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acid residues identical to those in the acceptor immunoglobulin heavy chain variable region framework.

112. A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

113. A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin

specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences;

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

114. (Amended) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs; [contains an atom within a distance of 6 Å of a CDR in said humanized immunoglobulin;]

and expressing the DNA segments in the cell to produce the immunoglobulin.

115. (Amended) A method of producing a pharmaceutical composition, comprising

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein the humanized immunoglobulin [antibody] was produced by:

(1) comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions;

(2) selecting a human heavy chain variable region framework from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region framework, wherein the selected variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor [human immunoglobulin] heavy chain variable region framework;

(3) synthesizing a DNA segment encoding a humanized heavy chain variable region, comprising complementarity determining regions (CDRs) [CDRs] from the donor immunoglobulin heavy chain variable region and a variable region framework from the selected acceptor heavy chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin heavy chain variable region and a DNA segment encoding a humanized immunoglobulin light [heavy] chain variable region into a cell; and

(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin.

Subsidiary

116. (Amended) A method of producing a pharmaceutical composition, comprising:

formulating [the] a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein

the humanized immunoglobulin was produced by a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework.

EB Cont'd

117. (Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein the humanized antibody has complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat [and Chothia] CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each

of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences and the replaced amino acid is rare at its position for human immunoglobulin sequences.

118. (Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition,

wherein the humanized antibody has complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy [or light] chain framework[s], and each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs [contains an atom within a distance of 6 Å of a CDR in said humanized immunoglobulin].

119. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to

an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, [or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences], wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

120. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of about 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences, wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

121. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of about 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, [contains an atom within a distance of 6 Å of a CDR in said humanized immunoglobulin]
wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

122. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs[, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences].

123. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor heavy chain immunoglobulin framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy [or light] chain framework[s], and each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

is capable of interacting with the CDRs [contains an atom within a distance of 6 Å of a CDR in said humanized immunoglobulin], or

(II) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences.

124. (Amended) A humanized immunoglobulin according to claim 120, wherein said donor amino acids are from the donor immunoglobulin heavy chain framework.

125. (Amended) A humanized immunoglobulin according to claim 121, wherein said donor amino acids are from the donor immunoglobulin heavy chain framework.

126. (Amended) A humanized immunoglobulin according to claim 122 or 123.

E3 concl'd

wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$[, wherein said amino acids are from the donor immunoglobulin heavy chain framework].

Please delete claims 127 ← 130.

Please amend the following claims:

Sub B3

E4

131. (Amended) A humanized immunoglobulin according to any one of claims 119, 121, or 122 [123 or 127-130], further comprising an amino acid from the donor immunoglobulin framework that replaces the corresponding amino acid in the acceptor immunoglobulin heavy or light chain frameworks, wherein said amino acid is typical at its position in human immunoglobulin sequences and said corresponding amino acid in the acceptor immunoglobulin is rare at its position in human immunoglobulin sequences.

132. A humanized immunoglobulin according to any one of claims 119 to 126 [130], which comprises at least 3 said amino acids from the donor immunoglobulin framework.

133. A humanized immunoglobulin according to any one of claims 119 to 126 [130], which comprises two light chain/heavy chain dimers.

134. A humanized immunoglobulin according to any one of claims 119 through 126 [130], which is substantially pure.

135. A pharmaceutical composition comprising a humanized immunoglobulin according to claim 134 in a pharmaceutically acceptable carrier.

Please add the following new claims:

Sub G3 cont

136. (New) A humanized immunoglobulin according to any one of claims 119-126, wherein said humanized immunoglobulin has an affinity for antigen up to about 10^{10} M^{-1} wherein said affinity is greater than the affinity of another immunoglobulin which has the same sequence as the humanized immunoglobulin except without the donor immunoglobulin framework amino acids that replace the corresponding amino acids in the acceptor immunoglobulin.

E5

137. (New) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids is not outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences;

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

138. (New) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32) and:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs;

and expressing the DNA segments in the cell to produce the immunoglobulin.

139. (New) A method of claim 112, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework.

140. (New) A method of claim 115, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework.

141. (New) A method of claim 116, wherein the sequence of the humanized